Phytoprotection

Phytotoxicity of *Fusarium*, other fungal isolates, and of the phytotoxins fumonisin, fusaric acid, and moniliformin to jimsonweed

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Volume 76, numéro 1, 1995

URI : https://id.erudit.org/iderudit/706081ar
DOI : https://doi.org/10.7202/706081ar

Aller au sommaire du numéro

Éditeur(s)

Société de protection des plantes du Québec (SPPQ)l

ISSN

0031-9511 (imprimé)
1710-1603 (numérique)

Découvrir la revue

Citer cet article

Abbas, H., Boyette, C. & Hoagland, R. (1995). Phytotoxicity of *Fusarium*, other fungal isolates, and of the phytotoxins fumonisin, fusaric acid, and moniliformin to jimsonweed. *Phytoprotection*, *76*(1), 17–25. https://doi.org/10.7202/706081ar

Résumé de l'article

Dix isolats fongiques isolés de la stramoine commune (*Datura stramonium*) et 7 isolats provenant d'espèces cultivées ont été examinés pour la production de phytotoxines et pour leur pouvoir pathogène sur des plantules de stramoine commune cultivées en serre. Quatre isolats de *Fusarium moniliforme*, trois isolatsde *F. semitectum*, un isolatde *F. oxysporum*, un isolatde *Cephalosporium spp*. et un isolât d'Alternaria crassa prélevés sur des plantules de stramoine commune infectées, et sept isolats supplémentaires de *F. moniliforme* obtenus de grains et de plantules d'espèces cultivées ont été mis en culture sur du riz (*Oryza sativa*) autoclave. Les mélanges champignon-riz ont été moulus et leur phytotoxicite sur des plantules de stramoine commune âgées de 1 et 2 semaines a été testée par des applications foliaires. Tous les extraits de riz infestés par un champignon (5 g de mélange riz-champignon 50 ml^{-1} d'eau) ont causé des dommages ou la mort des plantules, sauf les extraits d'isolats de *F. semitectum*, *Cephalosporium spp*. et *A. crassa*. Les mélanges champignon-riz ont été analysés de façon quantitative pour la présence de phytotoxines du *fusarium*[fumosinine B_1 (FB_1), acide fusarique et moniliformine]. Aucun isolât n'a produit plus d'une de ces phytotoxines dans les extraits de champignon-riz. La FB_1 était produite par tous les isolats de *F. moniliforme* isolés selon une échelle de concentration de $\leqslant$ 5 à 850 µg mL^{-1} de mélange champignon-riz. L'isolât de *F. oxysporum* a produit 3,5 g m L^{-1} de moniliformine et aucune phytotoxine n'a été détectée dans les extraits de *F. semitectum*, *Cephalosporium spp*. ou *A. crassa*. La fumonisine, l'acide fusarique et la moniliformine appliqués à l'état pur à du feuillage de stramoine commune à 6-50,25-800 et 50-800 µg mL^{-1} ont causé des symptômes similaires à ceux des isolats fongiques qui avaient produit ces composés. Des tests sur le pouvoir pathogène des spores de tous les isolats sur la stramoine ont indiqué que les isolats étaient avirulents, sauf *A. crassa* qui a causé des infections seulement après une durée d'humectation $\geqslant$ 12 h.

Phytotoxicity of *Fusarium*, other fungal isolates, and of the phytotoxins fumonisin, fusaric acid, and moniliformin to jimsonweed

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Received 1994-04-14; accepted 1995-06-20

Ten fungal isolates from jimsonweed (*Datura stramonium* L.) and 7 from crop species were examined for phytotoxin production and pathogenicity on jimsonweed seedlings in the greenhouse. Four isolates of *Fusarium moniliforme*, three *F. semitectum* isolates, a *F. oxysporum* isolate, a *Cephalosporium* spp. isolate, and an *Alternaria crassa* isolate from diseased jimsonweed seedlings, plus seven additional *F. moniliforme* isolates from seeds and seedlings of crop species were grown on autoclaved rice (*Oryza sativa*). The fungus-rice mixtures were ground and tested for phytotoxicity on 1- and 2-wk-old jimsonweed seedlings via foliar application. All fungus-infested rice extracts (5 g fungus-rice mixture 50 mL^{-1} water) caused injury or mortality to the seedlings except the extracts from isolates of *F. semitectum*, *Cephalosporium* spp., and *A. crassa*. Fungus-rice mixtures were quantitatively analyzed for the presence of *Fusarium* phytotoxins [fumonisin B_1 (FB_1), fusaric acid, and moniliformin]. No isolate produced more than one of these phytotoxins in the fungus-rice extract. FB_1 was produced by all *F. moniliforme* isolates in a concentration range of ≤ 5 to 850 $\mu g\ mL^{-1}$ of fungus-rice extract. The *F. oxysporum* isolate produced moniliformin at 3.5 g mL^{-1}, and no phytotoxins were detected in extracts of *F. semitectum*, *Cephalosporium* spp., or *A. crassa*. Pure fumonisin, fusaric acid, and moniliformin applied to jimsonweed foliage at 6-50, 25-800, and 50-800 $\mu g\ mL^{-1}$, respectively, caused symptoms similar to that of the fungal isolates that produced these compounds. Pathogenicity tests of spores of all isolates on jimsonweed indicated that the isolates were avirulent, except for *A. crassa* which infected only after a dew period ≥ 12 h.

Abbas, H.K., C.D. Boyette et R.E. Hoagland. 1995. Phytotoxicité du *Fusarium* et d'autres isolats fongiques, ainsi que des phytotoxines fumonisine, acide fusarique et moniliformine envers la stramoine commune. PHYTOPROTECTION 76: 17-25.

Dix isolats fongiques isolés de la stramoine commune (*Datura stramonium*) et 7 isolats provenant d'espèces cultivées ont été examinés pour la production de phytotoxines et pour leur pouvoir pathogène sur des plantules de stramoine commune cultivées en serre. Quatre isolats de *Fusarium moniliforme*, trois isolats de *F. semitectum*, un isolat de *F. oxysporum*, un isolat de *Cephalosporium* spp. et un isolat d'*Alternaria crassa* prélevés sur des plantules de stramoine commune infectées, et sept isolats supplémentaires de *F. moniliforme* obtenus de grains et de plantules d'espèces cultivées ont été mis en culture sur du riz (*Oryza sativa*) autoclavé. Les mélanges champignon-riz ont été moulus et leur

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phytotoxicité sur des plantules de stramoine commune âgées de 1 et 2 semaines a été testée par des applications foliaires. Tous les extraits de riz infestés par un champignon (5 g de mélange riz-champignon 50 mL^{-1} d'eau) ont causé des dommages ou la mort des plantules, sauf les extraits d'isolats de *F. semitectum, Cephalosporium* spp. et *A. crassa*. Les mélanges champignon-riz ont été analysés de façon quantitative pour la présence de phytotoxines du *Fusarium* [fumosinine B_1 (FB_1), acide fusarique et moniliformine]. Aucun isolat n'a produit plus d'une de ces phytotoxines dans les extraits de champignon-riz. La FB_1 était produite par tous les isolats de *F. moniliforme* isolés selon une échelle de concentration de ≤ 5 à 850 $\mu g\ mL^{-1}$ de mélange champignon-riz. L'isolat de *F. oxysporum* a produit 3,5 g mL^{-1} de moniliformine et aucune phytotoxine n'a été détectée dans les extraits de *F. semitectum, Cephalosporium* spp. ou *A. crassa*. La fumonisine, l'acide fusarique et la moniliformine appliqués à l'état pur à du feuillage de stramoine commune à 6-50, 25-800 et 50-800 $\mu g\ mL^{-1}$ ont causé des symptômes similaires à ceux des isolats fongiques qui avaient produit ces composés. Des tests sur le pouvoir pathogène des spores de tous les isolats sur la stramoine ont indiqué que les isolats étaient avirulents, sauf *A. crassa* qui a causé des infections seulement après une durée d'humectation $\geq$ 12 h.

INTRODUCTION

There is currently much interest in the use of various microbes and microbial products as weed control agents (Hoagland 1990a; TeBeest 1991). A current project in our laboratory involves the control of jimsonweed (*Datura stramonium* L.) utilizing various fungi, including several *Fusarium* species. Phytotoxicity of *Fusarium* species and their secondary metabolites have been well documented on field crops (Abbas *et al.* 1992; Burmeister and Plattner 1987; Datnoff and Sinclair 1988; Nelson 1992; Stack and McMullen 1985; Van Asch *et al.* 1992), fruits (Labuschagne *et al.* 1987; Timer 1982), and vegetables (Jones and Woltz 1981; Kuo and Scheffer 1964; Mirocha *et al.* 1992). Little has been reported on the phytotoxicity of *Fusarium* species and their secondary metabolites on weeds. One report (Abbas *et al.* 1991) showed that *F. moniliforme* (Sheldon) isolated from jimsonweed caused profound damage to jimsonweed and some other weed species. That isolate produced fumonisin B_1 (FB_1) in copious amounts and some related fumonisin compounds as minor metabolites (Abbas *et al.* 1992). FB_1 was also shown to be responsible for the fungal phytotoxicity to jimsonweed and other weeds (Abbas and Boyette 1992; Abbas *et al.* 1991; Duke *et al.* 1991; Tanaka *et al.* 1993). *Fusarium* species are well known for their production of phytotoxins such as fumonisins (Abbas *et al.* 1992; Gelderblom *et al.* 1988; Vesonder *et al.* 1990), fusaric acid (Abbas *et al.* 1989; Nelson *et al.* 1983), moniliformin (Abbas and Mirocha 1985; Nelson *et al.* 1983), enniatin (Burmeister and Plattner 1987), and trichothecenes (Abbas *et al.* 1989; Matsuo 1982).

Earlier tests with several *F. moniliforme* isolates from diseased jimsonweed indicated a lack of pathogenicity (Abbas *et al.* 1992). The present study was initiated to further study and compare the pathogenicity and phytotoxicity of various isolates of *Fusarium, Cephalosporium*, and *Alternaria crassa* (Sacc.) Rands from jimsonweed, and *Fusarium* from several crop species, when grown on solid or liquid media. We also sought to quantitate FB_1, fusaric acid, and moniliformin in fungus-rice (*Oryza sativa* L.) extracts of these fungi and to compare effects of fungal-rice extracts with those of high-purity forms of these three water-soluble toxins. Although FB_1 is produced mainly by *Fusarium* spp., this mycotoxin was recently reported to be produced in other fungal genera such as *Alternaria alternata* (Fries) Kiesler f. sp. *lycopersici* (Chen *et al.* 1992). *A. crassa* was originally isolated from jimsonweed and shown to be pathogenic to this weed (Boyette 1986), but its pathogenicity and phytotoxin production

had not been examined when cultured on solid media (rice grains) and when applied without dew. Because many of these organisms were isolated from diseased jimsonweed, an economically important weed in widespread areas of the world in soybean [*Glycine max* (L.) Merr.] and other crops (Mitich 1989), we used this weed as a bioassay species.

MATERIALS AND METHODS

Plant material

Jimsonweed seeds were mechanically scarified with sandpaper and planted in the greenhouse in a commercial potting mixture supplemented with a slow release N-P-K fertilizer (14-14-14). The plants were watered as needed, and the temperature maintained between 28 and 32°C at 40-60 % RH. The photoperiod was *ca* 14 h at 1600-1800 μmol m^{-2} s^{-1} (PAR) at midday. Treatments were applied when plants were 1- and 2-wk-old (2- to 4-leaf stage).

Fungal isolation

Fungal isolates and sources used in these studies are listed in Table 1. Isolates of *Fusarium* spp. (JW#1 and JW#3 to JW#9) and *Cephalosporium* spp. (JW#2) were obtained from stems and seed coats of infected jimsonweed plants grown in the greenhouse using procedures described by Abbas *et al.* (1989, 1991). The five *F. moniliforme* cultures, M-521, M-728, M-1271, M-5519 and M-5542 were isolated from Kentucky bluegrass (*Poa pratensis* L.), *Gladiolus* spp., rice, and corn, respectively. *F. moniliforme* M-5542B arose in our laboratory as a sector (morphological variant) from *F. moniliforme* M-5542. *A. crassa* was isolated from jimsonweed plants. *F. moniliforme* NRRL 18226 was isolated from corn (*Zea mays* L.) (Vesonder *et al.* 1990). Stock cultures of these fungi were maintained on an autoclaved skim milk-silica gel medium (Windels *et al.* 1988) stored at -4°C in the laboratory.

Inoculum production

The inoculum for pathogenicity studies was produced by growing the isolates on neutral-dox yeast solution (Lukens 1960) to facilitate spore production. Fungal mats and spores were separated from the liquid culture by filtration. These fungal propagules were washed with distilled water, refiltered, and homogenized with distilled water in an electric blender. These inocula preparations contained fungal spores, mainly macroconidia, at concentrations between 3 x 10^6 and 7.8 x 10^7 spores mL^{-1}, depending on the isolate. Spore concentrations were determined using a hemacytometer. These homogenized preparations were used directly as inocula.

The inoculum for phytotoxin analysis and phytotoxicity testing was produced by growing the isolates on a solid medium

Table 1. Sources of fungal isolates used

Fungal isolate	No. of isolates	Code	Source	Origin
Alternaria crassa[a]	1	NRRL–18136	Jimsonweed	Mississippi
Cephalosporium spp.	1	JW #2	Jimsonweed	Mississippi
Fusarium moniliforme[b]	1	M–521	Kentucky bluegrass	Pennsylvania
Fusarium moniliforme[b]	1	M–728	Gladiolus corms	Pennsylvania
Fusarium moniliforme[b]	1	M–1271	Rice seed	Pennsylvania
Fusarium moniliforme[b]	2	M–5519, M–5542	Corn seed	Pennsylvania
Fusarium moniliforme	1	M–5542B	Sector of M–5542	Mississippi
Fusarium moniliforme	1	NRRL–A28160	Corn seed	Illinois
Fusarium moniliforme	4	JW #1, 4, 5, 9	Jimsonweed	Mississippi
Fusarium oxysporum	1	JW #3	Jimsonweed	Mississippi
Fusarium semitectum	3	JW #6, 7, 8	Jimsonweed	Mississippi

[a] Fungus isolated from jimsonweed by Boyette (1986).
[b] Cultures purchased from the *Fusarium* Research Center Culture Collection, University Park, Pennsylvania.

consisting of converted long-grain enriched rice (Uncle Ben's Inc., Houston, Texas) as described by Abbas *et al.* (1991, 1992). Rice grains (200 g) plus 140 mL distilled water were autoclaved in flasks for 1 h (121°C, 15 psi) on two consecutive days, resulting in sterile, hydrated rice grains with a moisture content of 35-37 % (wt:wt). Individual flasks were inoculated with a given fungus and incubated at 22°C for 2 wk. The fungus-infested rice mixture was air-dried and ground into a powder. The powder (5 g) was added to 50 mL water for foliar spray application.

Pathogenicity tests

Fungal inocula from liquid culture were applied to leaves and stems of 1- and 2-wk-old (2- to 4-leaf stage) jimsonweed plants by spraying with an atomizer until runoff occurred. Control plants received distilled water. Plants were placed in a dew chamber for up to 24 h, then transferred to the greenhouse for assessment of infection over a 10-d period. Three replicates were used for each treatment. Each replicate contained 12 jimsonweed plants. The experiment was repeated twice.

Inocula from solid culture fungus-rice extracts, prepared as described above, were applied to leaves and stems of jimsonweed seedlings by spraying to runoff with an atomizer. Control plants received filtrates of autoclaved rice. Plants were placed in the greenhouse immediately after treatment. Treated and control plants were observed daily and symptoms were evaluated using a visual injury rating scale described below. The height of 6 jimsonweed plants was recorded at the beginning and end (2 wk) of each experiment. Three replicates, each containing 12 jimsonweed plants, were used for each treatment, and the experiment was repeated twice. Analogous experiments were carried out in which fungal-rice extracts were applied to jimsonweed plants, which were then placed in a dew chamber for 10-20 h before being placed in the greenhouse for 2 wk.

Injury or mortality determination

Injury to and mortality of jimsonweed seedlings by liquid-culture inocula, fungal-rice extracts, or purified phytotoxins was visually assessed 2 wk after treatment, using a scale based on that described by Hoagland and Boyette (1994). Injury was assigned a value of 0-4, where 0 = no injury (0 %) and 4 = severe chlorosis, necrosis, growth inhibition, wilt, or mortality (100 %). Ratings were combined averages of rating values for two observations of three replicates (composed of 6-10 seedlings) of each treatment. Percent mortality was determined 2 wk after treatment by direct counts of collapsed seedlings. Values of the replicates of each treatment were combined and averaged.

Phytotoxin standards

Standard samples of FB_1 were isolated and purified in our laboratories using high performance liquid chromatography (HPLC) and fast atom bombardment mass spectrometry (FAB-MS) methods described by Abbas *et al.* (1991, 1992) and Vesonder *et al.* (1990). Fusaric acid was purchased from Sigma Chemical Co. and moniliformin was provided by Dr. R. F. Vesonder.

Phytotoxicity of pure phytotoxins

To check the biological activity of the phytotoxins (FB_1, fusaric acid and moniliformin), intact plants were used as described in the section on pathogenicity tests. Various concentrations (6.3-50 µg mL^{-1} for FB_1 and 6.3-800 µg mL^{-1} for moniliformin and fusaric acid) were sprayed until runoff. Treated plants were then placed in the greenhouse. Dry weights of plant shoot material (biomass) were determined at the end of the experiment after excising stems at the soil line and drying for 48 h at 60-70°C.

Phytotoxin extraction and quantification

Procedures used for extraction, detection and determination of fusaric acid and moniliformin were described previously in Abbas *et al.* (1989). FB_1 was extracted from fungus-infested rice as described in Abbas *et al.* (1991, 1992) and purified as described in Vesonder *et al.* (1990). The fungus-infested rice (50 g) was extracted with 300 mL 60 % aqueous methanol and the extract purified by XAD-2, silica gel column chromatography and semi-preparative HPLC on C-18 reverse phase

silica (Vesonder *et al.* 1990). FB_1 was obtained as a white powder. Authenticity was confirmed by comparison with a standard of FB_1 from corn cultured with *F. moniliforme* MRS 825 (Gelderblom *et al.* 1988), using HPLC and FAB-MS techniques (Abbas *et al.* 1991, 1992; Vesonder *et al.* 1990).

RESULTS

Pathogenicity tests of the 17 liquid culture inocula followed by dew periods up to 24 h indicated that only *A. crassa* infected the young jimsonweed plants. The infectivity of *A. crassa* was expected, given its documented pathogenicity on this weed host (Boyette 1986). In the present tests, *A. crassa* from liquid culture (6.2×10^6 spores mL^{-1}) caused 100 % injury and high mortality (≥ 95 %) to 1- and 2-wk-old jimsonweed seedlings 2 wk after treatment. These tests were not designed to show possible interactions of phytotoxins with pathogenic effects of the fungal-rice extracts. However, in those cases where no phytotoxic injury was apparent (lack of phytotoxin production and infectivity), we were able to assess pathogenicity when fungal-rice extracts were applied to jimsonweed plants with or without a dew period. Fungal-rice extracts of three *F. semitectum* Berk. & Ravenel (JW #6, #7, and #8), the *Cephalosporium* spp., and the *A. crassa* isolate produced no injury and thus exhibited no pathogenicity without a dew period. Only one of these isolates, *A. crassa*, exhibited pathogenicity when a dew period of > 10 h was supplied. Ratings were 100 % injury and 85-95 % mortality on the young jimsonweed plants 2 wk after treatment.

Fungus-rice extracts were examined for phytotoxicity and for levels of FB_1, fusaric acid, and moniliformin. All *F. moniliforme* isolates produced FB_1 in a range of ≤ 5 to 850 μg mL^{-1} of fungus-rice extract (Table 2), but the other two phytotoxins were not detected. *F. oxysporum* (Schlect.) Synd. and Hans. from jimsonweed (JW #3) produced moniliformin as a major phytotoxin (3.5 g mL^{-1}), but did not produce FB_1 or fusaric acid. Rice extracts of the three *F. semitectum* isolates (JW #6, #7, and #8), one *Cephalosporium* spp. isolate from jimsonweed, and *A. crassa* contained no FB_1, fusaric acid, or moniliformin.

Table 2. Effects on growth of jimsonweed plants and phytotoxin production of *Fusarium* spp. isolates grown on a rice medium[a]

Fungal isolate	1-wk-old plants		2-wk-old plants		FB_1 (μg mL^{-1})
	Injury (%)	Mortality (%)	Injury (%)	Mortality (%)	
Controls					
Distilled water	0	0	0	0	–
Autoclaved rice extract	0	0	0	0	–
F. moniliforme					
JW #1	100	100	100	89 ± 21.3	850
JW #4	100	100	100	33 ± 9.5	420
JW #5	100	95 ± 19.3	100	11 ± 4.5	340
JW #9	100	90 ± 17.2	100	19 ± 3.7	315
M-521	100	0	100	6 ± 2.2	≤ 5
M-728	100	0	90 ± 13.5	0	≤ 5
M-1271	25 ± 6.3	0	20 ± 9.5	0	≤ 5
M-5519	100	0	80 ± 13.4	0	≤ 5
M-5542	100	0	85 ± 11.2	0	≤ 5
M-5542B	100	95 ± 15.7	100	17 ± 5.2	345
NRRL-A28160	100	10 ± 5.2	100	0	≤ 5
F. oxysporum					
JW #3	80 ± 17.5	0	100	0	0

[a] *A. crassa, Cephalosporium* spp. and *F. semitectum* produced no injury symptoms or detectable levels of FB_1, fusaric acid, or moniliformin. Results are the mean of three replicates for each treatment ± one standard error.

Assessment of the effect of these fungal-rice extracts on jimsonweed seedlings showed a strong positive relationship between injury or mortality and the levels of phytotoxin produced (Table 2). Isolates could generally be grouped into several categories based on the extent of injury or mortality they exhibited. Isolate JW #1 caused the highest injury and mortality to 1- and 2-wk-old jimsonweed seedlings, and it also produced the highest level of FB_1 (850 μg mL^{-1}). Isolates M-5542B, JW #9, JW #5, and JW #4 caused less injury and mortality and produced less FB_1 (345-420 μg mL^{-1}). A third group was comprised of isolates M-521, M-728, M-5519, and M-5542, and NRRL-A-28160, which caused high injury but no mortality; these fungi produced FB_1 at ≤ 5 μg mL^{-1}. Isolate M-1271 produced the lowest levels of FB_1, and caused the lowest injury and no mortality. Fungal-rice extracts from the three *F. semitectum* isolates (JW #6, #7 and #8), the *Cephalosporium* spp. isolate (JW #2), and *A. crassa* (NRRL-18136), all lacking production of FB_1, fusaric acid, or moniliformin, did not cause injury to jimsonweed.

High-purity FB_1, fusaric acid, and moniliformin were applied to jimsonweed seedlings, and effects on plant growth, injury, and mortality determined (Table 3). Pure FB_1 at 6.3, 12.5, 25, and 50 μg mL^{-1} was highly phytotoxic to jimsonweed plants. The symptoms caused by this phytotoxin were similar to those caused by the fungus-rice extracts of isolates that produced FB_1. Pure moniliformin applied at 50-800 μg mL^{-1} caused symptoms similar to the fungus-rice extract from JW #3 (Table 3). None of the fungal isolates produced detectable levels of fusaric acid when grown on rice (Table 2). Nevertheless, this compound was more phytotoxic than moniliformin.

Table 3. Effects of various rates of application of three phytotoxins on the growth and biomass production of 2-wk-old jimsonweed plants[a]

Phytotoxin	Application rate (μg mL^{-1})	Plant height (% reduction)	Dry wt (% reduction)	Injury (%)	Mortality (%)
Control (distilled water)	0	0	0	0	0
FB_1	6.3	89 ± 16.2	78 ± 11.5	100	5 ± 1.2
	12.5	ND[b]	ND	100	37 ± 4.5
	25	ND	ND	100	92 ± 20.5
	50	ND	ND	100	100
Fusaric acid	6	6 ± 3.3	0	0	0
	12.5	23 ± 5.3	0	0	0
	25	35 ± 5.3	24 ± 6.4	80	0
	50	34 ± 7.7	38 ± 7.3	100	0
	100	34 ± 11.2	52 ± 10.2	100	0
	200	33 ± 13.5	55 ± 17.2	100	0
	400	ND	ND	100	3.5 ± 7.2
	800	ND	ND	100	50 ± 8.5
Moniliformin	6	0	0	0	0
	12.5	2 ± 1.2	0	0	0
	25	19 ± 3.4	5 ± 3.3	10 ± 3.5	0
	50	40 ± 10.3	42 ± 7.8	77 ± 15.5	0
	100	43 ± 10.3	48 ± 7.8	100	0
	200	51 ± 14.5	55 ± 10.2	100	0
	400	55 ± 18.2	60 ± 15.2	100	0
	800	58 ± 20.2	62 ± 14.5	100	0

[a] Results are the means of three replicates ± one standard error.
[b] ND = Not determined due to mortality.

DISCUSSION

Several *F. moniliforme* isolates caused substantial injury to jimsonweed plants when their fungal-rice culture extracts were applied to jimsonweed foliage. This was positively related to the production of the highly active phytotoxin FB_1. Damage varied depending on the amount of toxin produced by the isolate and on the production of metabolites related to FB_1. These results support other findings (Abbas *et al.* 1991, 1992). This study also showed that fungal-rice extracts of the *F. moniliforme* isolates obtained from jimsonweed were generally more phytotoxic than those obtained from other sources, which also correlates with damage caused to jimsonweed plants by culture filtrates (Abbas *et al.* 1991, 1992). Extracts from the *F. oxysporum* isolate contained high levels of moniliformin and caused severe injury to jimsonweed. The phytotoxicity of moniliformin to crop and weed species is well documented (Abbas *et al.* 1991; Hoagland 1990b; Vesonder *et al.* 1992). Rice culture extracts of *F. semitatum, A. crassa,* and *Cephalosporium* spp. were not injurious to jimsonweed when applied without a dew period, apparently because they contained no phytotoxins. A recent study of the relationship of pathogenicity and phytotoxin production in 50 *Fusarium* species isolated from red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.) was also reported (Nedelnik 1992). Pathogenicity of the isolates was similar in the two plant species, but phytotoxins in fungal–culture filtrates were generally more toxic to clover. None of the phytotoxins in that study were identified.

Preparations of spores and mycelia from liquid cultures of *Fusarium* or *Cephalosporium* isolates did not infect jimsonweed, even when long dew periods (10-24 h) were used to promote propagule germination and growth on leaf surfaces. This suggests that these fungi are not pathogenic to jimsonweed, but can cause injury and mortality via the production of phytotoxic metabolites when grown on solid media such as rice. Although *A. crassa* did not produce any of these three phytotoxins, fungal-rice extracts of this jimsonweed pathogen did infect and cause injury and mortality to jimsonweed seedlings, but only if supplemented with a dew period of at least 10-12 h. This dew period requirement is similar to that previously reported for this pathogen when grown on liquid media (Boyette 1986). Growth and phytotoxin production can vary with species and the choice of liquid or solid media, as reported by Alberts *et al.* (1993). For example, *F. moniliforme* isolate NRRL-A28160 produced 460 $\mu g\ mL^{-1}$ FB_1 when grown on corn meal (Vesonder *et al.* 1990), but produced $\leq 5\ \mu g\ mL^{-1}$ on rice (Table 2).

In some cases, the injury and mortality observed in plants treated with fungal-rice extracts were much lower than anticipated (based on phytotoxin content) when compared to the effects of the pure phytotoxins. Some of these isolates may have produced stereochemical isomers detected as FB_1 but which are not as active as FB_1. Studies on the absolute and relative stereochemical configuration of FB_1 indicates numerous possible isomers (Hoye *et al.* 1994). How the biological activity of FB_1 varies with changes in stereochemistry is presently unknown. Constituents in fungal-rice extracts (starch, protein, polymers, etc.) could have prevented entry of a phytotoxin into the cuticle and plant cell walls by binding, rendering it ineffective. Such binding on foliar surfaces could prolong exposure of the phytotoxin to photo- and thermal-degradation. As an example, the *F. oxysporum* isolate produced high levels of moniliformin and some injury, but caused no mortality. Other studies have shown that moniliformin in ground corn and wheat exhibited a loss of 15 % in samples stored at 22°C for 150 h, and heating at 50°C for 2 h caused a 15 % and 40 % loss in corn and wheat, respectively (Scott and Lawrence 1987). Another problem with moniliformin is that its extraction and recovery are inconsistent in some instances (Scott *et al.* 1986), which could implicate binding and other factors. FB_1 thermostability is somewhat greater than that of moniliformin, but some degradation occurs. FB_1 levels in *F. moniliforme* dried corn cultures were reduced by 17 % after exposure to 75°C for 135 min (Dupuy *et al.* 1993). Greenhouse temperatures and sunlight could have

caused substantial degradation of labile phytotoxins in our foliar-applied samples.

Although some of the secondary products of *Fusarium* spp. are potent phytotoxins, many of these products also exhibit mammalian toxicity. For example, FB_1 is a known mammalian toxin, causing equine leukoencephalomalacia in horses and pulmonary edema in swine (Riley *et al.* 1993). It is, however, possible that analogs could be found with minimal mammalian toxicity and maximal herbicidal activity. This potential exists especially due to the large number of stereochemical isomers of FB_1 (Hoye *et al.* 1994) and because other fumonisins (FB_2, FB_3, and FB_4) produced by *Fusarium* spp. have varied phytotoxicities. Similarly, numerous analogs of moniliformin have been synthesized in an effort to obtain herbicidal products (Hoagland 1990b). Further research on the phytoxicity of fumonisin analogs is being conducted.

ACKNOWLEDGEMENTS

The moniliformin used in this study was generously provided by Dr. R.F. Vesonder, USDA-ARS, National Center for Agricultural Utilization Research, Peoria, Illinois. *Fusarium* species were identified by Paul E. Nelson, *Fusarium* Research Center, The Pennsylvania State University, Pennsylvania.

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